



Application No.: 10/828,642 Inventor(s): Schaffner, Richard I.

Filed: April 21, 2004; Art Unit: 1651 Examiner: Ford, Allison

Docket No.: SR565-OXID

Title: Oxidant-Scavenging Compound for Anaerobic Treatment

**DECLARATION UNDER 37 C.F.R. 1.132**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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Alexandria, VA 22313-1450

I, Richard I. Schaffner, declare as follows:

1. I am familiar with the subject matter of the above-identified application. I am particularly familiar with the fields of environmental remediation and bioremediation.

2. I have a BS (1987) in Geology from Brigham Young University (BYU) and an MS (2001) in Environmental Engineering from Kennedy Western University. I carried out graduate studies in Contaminant Hydrogeology at BYU from 1988 to 1992. I have worked in contaminant hydrogeology and remedial engineering for over 15 years, and am currently a Senior Technical Specialist with GZA GeoEnvironmental, Inc. in Manchester, NH. I have published numerous papers in the peer reviewed literature on biological remediation technologies. I also moderate the Bioremediation Discussion Group on the Internet ([www.bioremediationgroup.org](http://www.bioremediationgroup.org)), a Listserver serving some 5,000 environmental scientists/engineers worldwide who participate in environmental biotechnology transfer.

The analytical work was performed at Eastern Analytical, Inc. (EAI; [www.eailabs.com](http://www.eailabs.com)) of Concord, NH. They are a full-service environmental chemistry laboratory that has been in business for about 25 years. They were certified for BOD analysis in around 1989 through the National Environmental Laboratory Accreditation Conference (NELAC), and for DOC in

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around 2003 also through NELAC. EAI is certified for these analyses in all six New England states. A copy of their certification is available at their web site.



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3. Under my direction, experiments were performed to empirically evaluate the functional differences between Brewer's Yeast and Yeast Extract. Two different analytical laboratory tests were performed in accordance with United States Environmental Protection Agency (USEPA) standard methods<sup>1</sup>. Each test was performed using equivalent mass/volume concentrations of Brewer's Yeast or Yeast Extract at time zero (i.e., 500 milligrams per liter, mg/L, which is below the aqueous solubility limit for either yeast). The first test was modified 5-Day Biochemical Oxygen Demand (BOD5) performed in accordance with USEPA Standard Method 5210B. The second test was Dissolved Organic Carbon (DOC) performed in accordance with USEPA Standard Method 5310C.

4. Results of the modified BOD5 analyses were that 500 mg/L Brewer's Yeast and 500 mg/L Yeast Extract yielded the following BOD concentrations for the three time steps (Days 1, 2, and 5):

DAY	ESTIMATED BOD, mg/L	
	BREWER'S YEAST	YEAST EXTRACT
1	50	200
2	170	240
5	250	360

As shown on the table, Yeast Extract posed higher BOD for each time step than Brewer's Yeast on an equivalent mass basis. For example, Day 1, Day 2, and Day 5 BOD results for Yeast Extract respectively yielded 300%, 41%, and 44% more BOD than for Brewer's Yeast (percent increase in BOD for Brewer's Yeast relative to Yeast Extract). The magnitudes of these percent differences, ranging from 41% to 300% more BOD for Yeast Extract than Brewer's Yeast for equivalent time steps and time zero yeast concentration, is directly consistent with the position that the substitution of Brewer's Yeast preserves the nutritional value of the yeast such that it is not used up immediately upon hydration. In addition, the data trend is also consistent with this beneficial attribute of Brewer's Yeast—the percent difference for the Day 1 time step (300%) is an order of magnitude greater than for the Day 2 and 5 time steps (41% and 44%, respectively), suggesting that significantly more Yeast Extract is

<sup>1</sup> American Water Works Association, 1999, 20th Edition of Standard Methods for the Examination of Water and Wastewater.

<sup>2</sup> The only modification to the BOD5 analysis was that in addition to estimating BOD5 for Day 5, BOD was also estimated for Days 1, 2, and 5.



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bioavailable initially than Brewer's Yeast which is directly consistent with the applicant's position that Brewer's Yeast is significantly different and better than Yeast Extract for the desired use.

The following table summarizes modified BOD5 results as ratios (mass/volume basis) of BOD to time zero yeast concentrations (BOD Ratios) for the different time steps:

DAY	BOD RATIO (% Mass/Volume Basis)	
	BREWER'S YEAST	YEAST EXTRACT
1	10%	40%
2	34%	48%
5	50%	72%

As shown in this chart, while about 40% of the Yeast Extract was bioavailable for the initial Day 1 time step, only about 10% of the Brewer's Yeast was bioavailable for that time step under test conditions, for a net difference of about 30% more bioavailable Yeast Extract (i.e., difference between 40% BOD Ratio for Yeast Extract, and the 10% BOD Ratio for Yeast Extract). For Days 2 and 5, Brewer's Yeast also yielded lower BOD Ratios than Yeast Extract for each time step, though net differences were less than for Day 1 (i.e., 14% more Yeast Extract for Day 2, and 22% more for Day 5). The significantly greater lower BOD Ratio of Brewer's Yeast (10%) to Yeast Extract (40%) for the Day 1 time step relative to the later time steps suggests that significantly more Yeast Extract is bioavailable initially than Brewer's Yeast, directly consistent with the position that Brewer's Yeast is significantly different and better than Yeast Extract for the desired use.



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5. Results of the DOC analyses were that 500 mg/L Brewer's Yeast yielded about 50 mg/L DOD, whereas 500 mg/L Yeast Extract yielded about 170 mg/L DOC. Therefore, for the same unit mass of yeast, Yeast Extract yielded 240% more BOD than Brewer's Yeast. As bioavailability is directly linked to aqueous solubility, the fact that Yeast Extract yielded significantly more DOC than Brewer's Yeast is consistent with lower initial bioavailability than Yeast Extract, consistent with the applicant's claim. DOC Ratios for the two yeasts (i.e., ratios of DOC to time zero concentrations) are as follows:

$\Sigma$  10% for the Brewer's Yeast; and

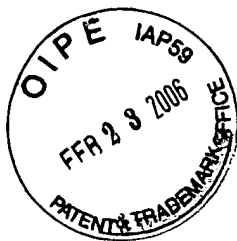
$\Sigma$  34% for the Yeast Extract.

The greater DOC ratio for Yeast Extract, relative to Brewer's Yeast, is consistent with Brewer's Yeast yielding significantly less DOC than Yeast Extract. As discussed previously, the full nutritional value of the Brewer's Yeast can only become available once the cell walls of the dead yeast cells are lysed.

6. The converging lines of evidence from the modified BOD5 and DOC analytical laboratory analyses show that Brewer's Yeast is surprisingly different and statistically better than Yeast Extract for use in reductive dehalogenation, and are directly consistent with the applicant's claim that Brewer's Yeast is a novel and substantive improvement over Yeast Extract because it preserves more of the nutritional value of the yeast for supporting reductive dehalogenation.

Further deponent sayeth not.

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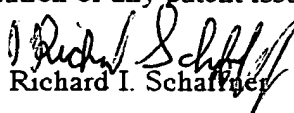
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This Declaration is made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 USC 1001, and may jeopardize the validity of the above-captioned patent application or any patent issuing thereon.

  
Richard I. Schaffner

1/20/06

Date

18 USC 1001: Whoever, in any matter within the jurisdiction of any department or agency of the United States knowingly and willingly falsifies, conceals or covers up any trick, scheme, or advice a material fact, or makes any false, fictitious or fraudulent statements or representations, or makes or uses any false writing or document knowing the same to contain any false, fictitious or fraudulent statement or entry, shall be fined not more than \$10,000 or imprisoned not more than five years or both.

*i.e.*, on the order of about 10%<sub>weight</sub>, about an order of magnitude more soluble than Brewer's Yeast.

2 American Water Works Association, 1999, 20th Edition of Standard Methods for the Examination of Water and Wastewater.

The only modification to the BOD<sub>5</sub> analysis was that in addition to estimating BOD<sub>5</sub> for Day 5, BOD was also estimated for Days 1, 2, and 5.